Chronic DARPP Nuclear Retention of Type of Antipsychotic

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Introduction

Antipsychotic drugs (APDs) have been the primary treatment for the positive symptoms of schizophrenia for over fifty years. Although these drugs were found to block dopamine D2 receptors, the downstream mechanisms of therapeutic action remain unclear. Furthermore, APDs usually produce potentially irreversible side effects (including movement and metabolic disorders), indicating the need for a more targeted approach.

Both D1 and D2 dopamine receptors are highly expressed in the striatum. However, D1 and D2 receptors generally do not colocalize on the same striatal neurons, and also exert opposing effects on downstream signaling. Cell-type specific effects can be masked in extracts of whole striatum.

Transgenic mice expressing differentially tagged proteins in D1 versus D2 neurons allow cell-type specific analysis of downstream signaling elements of the dopaminergic pathway (Bateup et al. 2008), such as the striatal enriched phosphoprotein DARPP-32 (Dopamine and Adenosine Regulated Phosphoprotein of 32 KDa).

DARPP-32 was found to be a potent inhibitor of Protein Phosphatase 1 (PP1) when phosphorylated on the threonine 34 (T34) residue. More recently (Stipanovich et al. 2008), dephosphorylation of the serine 97(S97) residue of DARPP-32 was found to promote DARPP-32 nuclear retention and accumulation.

Mutation of the S97 residue was found to impair dopamine regulated responses and histone phosphorylation states (Stipanovich et al. 2008). The aim of this project is to elucidate the effects of antipsychotic drugs on the phosphorylation and intracelluar localization of DARPP-32. We further plan to assess the role of nuclear DARPP-32 on histone modifications and transcriptional regulation.

Methods

Animals: 10-12 week old doubly transgenic (D1−/−D2−/−D1−/−D2−/−Flag: D1−/−D2−/−Myc) were received from the Greengard lab (Rockefeller University) and bred in the Yale Animal Facility. Acute drug groups were given a single intraperitoneal injection of either drug or vehicle (vehicle) and sacrificed 15 minutes later by microwave exposure. Chronic drug groups received daily intraperitoneal injections for 21 days, and sacrificed 24 hours after the final dose. Care and use of animals was in compliance with IACUC (Institutional Animal Care and Use Committee) recommendations for ethical treatment of animals.

Drugs: Haloperidol, Clozapine, and Risperidone were purchased from Sigma and used in the following concentrations: Haloperidol (1mg/kg), Clozapine (5mg/kg), and Risperidone (1mg/kg). All drugs were dissolved in 0.9% saline except Risperidone which was dissolved in 1% acetic acid for solubility, and the pH was adjusted back up to 6 with sodium hydroxide. Vehickle contained acetic acid and was adjusted to the same pH as the soluton with an equal volume of 0.9% saline.

Tissue Preparation and Immunoprecipitation: Mice were sacrificed by focused microwave irradiation and then refrigerated on ice. Tissues were homogenized in 50µL of 50mM potassium acetate, centrifuged for 5 minutes at 10000 rpm. An aliquot of total cellular homogenate was separated from each sample and the remaining homogenate was added to 50µL flag agarose beads (Sigma EZView red) and rotated overnight at 4°C. Flag beads were washed three times and eluted in 5µL sample buffer. Even though we were removing bead eluates from the samples there was still some remaining bead material. Flag antibodies were washed according to the manufacturer’s instructions and the remaining beads were added to 50µL slurry Myc-conjugated sepharose beads (Cell Signaling), and rotated overnight at 4°C until processing.

Samples from total, flag, and myc extracts were loaded on 4-20% tris-glycine midi gels (Life Technologies) and transferred overnight to PVDF membrane. Membranes were blocked for one hour with 5% non-fat dry milk and overnight with primary antibody (pS97 DARPP-32 Ab(1:1000, Greengard Lab), anti-Rabbit IgG HRP-conjugated secondary (1:2000, Pierce) for one hour at room temperature. Blots were developed with WestChem ECL solution (Pierce) and imaged using the BioRad ChemiDoc. Membranes were immunostained with pT34 DARPP-32 (1:1000, Cell Signaling). Blots were stripped and re-probed with a pT34 DARPP-32 antibody (1:1000, Cell Signaling). Blot were stripped again and re-probed with a pT34 DARPP-32 antibody (1:1000, Cell Signaling).

Analysis: All bands were quantified using ImageJ and normalized to normalizing total DARPP-32 value by sample. Normalized values were adjusted to the average of normalized control values (this was carried out to avoid biasing totalextracts were normalized to total control). Flag in flag control, ratio to myc control and plotted using GraphPad Prism. Values were excluded as outliers if they varied by greater or less than two standard deviations from the mean. Significance was calculated by t-test and marked as significant with p-value less than 0.05.

Conclusions

• Acute haloperidol decreased pS97 phosphorylation only in D2 neurons.
• Acute Clozapine decreased pS97 in total extracts, D1, and D2 fractions.
• Acute Risperidone decreased pS97 in total extracts and D2 fractions.

• Chronic haloperidol decreased pS97 in total extracts and D2 fractions.

• Chronic haloperidol, clozapine, and risperidone treatment has no significant effect on pS97 and varied effects on pT34.

References


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